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ISOLATION AND CHARACTERIZATION OF A FULL-LENGTH KNOTTED GENE FROM OIL PALM (ELAEIS GUINEENSIS JACQ.)

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By

CHE RADZIAH BT. CHE MOHD. ZAIN

Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia, in Fulfilment of the Requirement for the Degree of Doctor of Philosophy

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Chairman: Ho Chai Ling, PhD

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The oil palm is one of the most important commercial oil crops that produces high quality of oil and with it comes an increasing global demand for this crop. The clonal oil palm produced by tissue culture technique provides an opportunity to expand productivity in order to fulfill the vast demand. However a number of reports have documented the occurrence of abnormalities in clonal oil palm such as vegetative abnormalities of tissue-cultured plantlets and also abnormalities of flowers. There has been no fundamental understanding of the cause of these abnormalities. Since the tissues and organs of plants originate from apical meristems, it is worthwhile to study the molecular mechanism underlying organ morphogenesis from this undifferentiated meristem cells. Within the past several years, there are several lines of evidence indicating that the homeobox genes control pattern formation and morphological structure determinations in multicellular eukaryotes including plants. In this study, *Knotted* homeobox gene was identified and isolated in order to develop an understanding of the function of this gene during oil palm development. In order to isolate the full-length *Knotted* homeobox gene

from oil palm, an oil palm (*Elaeis guineensis* Jacq.) suspension culture cDNA library of was screened with a partial cDNA clone (putative oil palm *Knotted* gene). From the screening, the full-length of oil palm *Knotted* homeobox gene was isolated and designated as oil palm Knotted homeobox gene (*OPKN1*). This thesis describes the first homeobox gene isolated from oil palm. Sequence analysis showed that *OPKN1* belongs to the class-1 *Knotted* gene family. Expression study by semi-quantitative RT-PCR, northern blot as well as *in situ* hybridization analyses proved that *OPKN1* gene was expressed in both vegetative and floral meristems suggesting a function in both phases of development. Based on semi-quantitative RT-PCR analyses, *OPKN1* was shown to be expressed in all meristem–based tissues including shoot apex, both male and female floral meristems, embryogenic callus, and suspension cell cultures. This seems to indicate that *OPKN1* may play role in meristem organization and later in morphogenesis processes. This can be supported by the presence of *OPKN1* transcript in leaf primordia suggesting that it may play roles in the development of pinnate leaf in oil palm. The presence of *OPKN1* throughout the flower development in both normal and abnormal flowers also indicates that the gene is active during floral development, suggesting a function in floral morphogenesis. Furthermore, the differences in temporal and spatial expression of *OPKN1* in different stages and type of meristem tissues may cause error in organ determination.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Doktor Falsafah

PEMENCILAN DAN PENCIRIAN JUJUKAN LENGKAP *KNOTTED* GEN DALAM KELAPA SAWIT (*ELAEIS GUINEENSIS* JACQ.)

Oleh

CHE RADZIAH CHE MOHD ZAIN

Julai 2005

Pengerusi: **Ho Chai Ling, PhD**

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Kelapa sawit adalah salah satu daripada tanaman komersial penting yang menghasilkan minyak yang bermutu tinggi. Ini menyebabkan peningkatan global terhadap permintaan tanaman sawit. Kelapa sawit klonal yang dihasilkan melalui teknik kultura tisu menberi peluang bagi meningkatkan produktiviti bagi memenuhi permintaan yang pesat ini. Walau bagaimanapun, terdapat laporan-laporan yang melaporkan kelapa sawit klonal yang luarbiasa dari segi vegetatif dan pembungaan. Sehingga kini, sebab kejadian luar biasa ini masih tidak difahami secara asas. Oleh kerana tisu dan organ tumbuhan berasal daripada meristem apical, maka adalah berguna untuk mengkaji mekanisma molekular yang bertanggungjawab untuk morfogenesis daripada sel meristem yang belum membeza. Sejak beberapa tahun yang lepas, terdapat bukti-bukti yang menunjukkan bahawa gen 'homeobox' mengawal pembentukan dan morfologi struktur dalam pelbagai eukariot termasuk tumbuhan. Dalam kajian ini, gen 'Knotted homeobox' telah pun dikenalpasti dan dipencil untuk pemahaman fungsi gen ini semasa perkembangan kelapa sawit.

Untuk pemencilan jujukan lengkap gen *Knotted* homeobox daripada kelapa sawit, penyaringan perpustakaan cDNA kultur sel suspensi kelapa sawit dijalankan dengan menggunakan klon cDNA separa lengkap. Daripada penyaringan ini, jujukan lengkap gen *Knotted* homeobox telah dipencilkan dan dinamakan sebagai 'oil palm Knotted homeobox gene' (*OPKN1*). Tesis ini menerangkan gen homeobox buat pertama kalinya daripada kelapa sawit. Analisis jujukan menunjukkan bahawa *OPKN1* berasal daripada keluarga gen 'Knotted class I'. Kajian penzahiran menggunakan analisis RT-PCR separa kuantitatif, 'northern blot' dan '*in situ* hybridization' membuktikan bahawa *OPKN1* dizahirkan di dalam meristem pucuk dan bunga, mencadangkan fungsinya di dalam kedua-dua fasa perkembangan tersebut. Berdasarkan kepada analisis RT-PCR separa kuantitatif, *OPKN1* dizahirkan di dalam semua tisu berasaskan meristem termasuk apeks pucuk, meristem bunga jantan dan betina, kalus embriogenik dan kultur sel suspensi. Ini menunjukkan *OPKN1* memainkan peranan di dalam organisasi meristem dan kemudian proses morfogenesis. Fakta ini disokong dengan kehadiran transkrip *OPKN1* di dalam primordia daun lalu mencadangkan ia mungkin berperanan untuk perkembangan daun pinate kelapa sawit. Kehadiran *OPKN1* sepanjang perkembangan bunga normal dan luar biasa juga menunjukkan bahawa gen ini aktif semasa perkembangan bunga, lalu mencadangkan fungsinya di dalam morfogenesis bunga. Tambahan pula, perbezaan penzahiran secara 'temporal dan spatial' *OPKN1* di dalam tahap dan jenis tisu meristem mungkin menyebabkan kesilapan dalam penentuan organ.

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Universiti Putra Malaysia, Nov 2004.

I certify that an Examination Committee met on 21th July 2005 to conduct the final examination of Che Radziah bt. Che Mohd. Zain on her degree in Doctor of Philosophy thesis entitled "Isolation and Characterization of a Full-length *Knotted* Gene from Oil palm (*Elaeis guineensis* Jacq.)" in accordance with Universiti Pertanian Malaysia (Higher Degree) Act 1980 and Universiti Pertanian Malaysia (Higher Degree) Regulations 1981. The Committee recommends that the candidate be awarded the relevant degree. Members of the Examination Committee are as follows:

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DECLARATION

I hereby declare that the thesis is based on my original work except for equations and citations, which have been duly acknowledged. I also declare that it has not been previously or concurrently submitted for any other degree at UPM or other institutions.

CHE RADZIAH BT. CHE MOHD. ZAIN

Date: 29 OCT 2004

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BCIP	-	5-bromo-4-chloro-3-indolyl phosphate
bp	-	basepair
kbp	-	kilobasepair
BSA	-	bovine serum albumin
cfu	-	colony forming unit
CTAB	-	hexadecyl (or cetyl) trimethyl ammonium bromide
dATP	-	deoxyadenine triphosphate
dCTP	-	deoxycytosine triphosphate
dTTP	-	deoxythymine triphosphate
dGTP	-	deoxyguanine triphosphate
ATP	-	adenine triphosphate
CTP	-	cytosine triphosphate
UTP	-	uracil triphosphate
GTP	-	guanine triphosphate
DEPC	-	diethyl pyrocarbonate
DIG	-	Digoxigenin
DMSO	-	dimethyl sulphoxide
DNA	-	deoxyribonucleic acid
cDNA	-	complemetary deoxyribonucleic acid
DNase	-	nuclease
DTT	-	dithiothreitol
EDTA	-	ethylene diamine tetracetate
g	-	gram
mg	-	milligram
µg	-	microgram
HCl	-	hydrochloric acid
LB	-	Luria-Bertani
LiCl	-	lithium chloride
M	-	molar / molarity
mM	-	millimolar
µM	-	micromolar
$MgSO_4$	-	magnesium sulfate
ml	-	milliliter
µl	-	microliter

N	-	Normality
NaCl	-	sodium chloride
NaOH	-	sodium hydroxide
NBT	-	nitroblue tetrazolium chloride
ng	-	nanogram
NTE	-	NaCl-Tris-EDTA
OD	-	optical density
PBS	-	phosphate buffer saline
PCR	-	Polymerase Chain Reaction
pfu	-	plaque forming unit
pmole	-	picomole
RNA	-	ribonucleic acid
mRNA	-	messenger ribonucleic acid
rRNA	-	ribosomal ribonucleic acid
RNase	-	ribonuclease
rpm	-	revolution per minute
RT	-	Reverse Transcriptase
SAAP	-	streptavidin-alkaline phosphatase conjugate
SDS	-	sodium dodecyl sulfate / sodium lauryl sulfate
SSC	-	standard saline citrate
TBS	-	tris buffer saline
TCA	-	trichloroacetic acid
TE	-	Tris-EDTA
Tris	-	tris[hydroxymethyl]aminomethane
Tris-HCl	-	tris hydrochloride
U	-	unit
UV	-	ultraviolet
V	-	volt
v/v	-	volume per volume
w/v	-	weight per volume
X	-	times
OPKN1	-	Oil palm *Knotted* homeobox gene
OPHb1	-	Oil palm homeobox1 partial cDNA clone

CHAPTER 1

INTRODUCTION

"He who sees how things grow from the very beginning will have the finest view of them"

-Aristotle-

How plants grow and propagate has been the subject of interest ever since human beings and plants bump into each other. This is spurred even further in the last few decades with the advances in molecular biology, leading to the development of sophisticated techniques in plant developmental study. Plants have two basic growth modes- vegetative growth and reproductive growth. Plants grow and develop new organs from a group of self perpetuating cells called meristems, normally at the growing shoot tips and root tips through the process of growth and differentiation. This eventually will give rise to different mature structure- the meristemic cells at the root tip will produce the root structure, while those at the shoot tip will initially produce stem and leaves before growing the thorns, buds and flowers (Kerstetter and Hake, 1997). One of the most important questions in biology is about the molecular mechanism that underlies the developmental regulation of the shoot apical meristem, and the initiation and subsequent differentiation of lateral organs.

It has been discovered that homeodomain proteins are involved in controlling a range of developmental processes (Gehring *et al.*, 1994; Chan *et al.*, 1998; Williams, 1998). Homeodomains are protein segments of approximately 60 amino acid residues that are encoded by DNA fragments called homeobox (Otting *et al.*, 1990). Mutational and evolutionary analysis is beginning to pinpoint specific roles of homeobox gene in plant meristem function. Several reports have indicated that homeobox genes control pattern formation and determine morphological structure in multicellular eukaryotes including plants (Sundas-Larson *et al.*, 1998). Previous studies have shown that *Knotted* genes encode for homeodomain proteins. It was also recorded that this family of genes cause altered morphology in rice, maize, tobacco and *Arabidopsis* (Matsuoka *et al.*, 1993; Sinha *et al.*, 1993; Tamaoki *et al.*, 1997; Chuck *et al.*, 1996). These observations suggested that *Knotted* genes might be involved in the development and morphogenesis of plants.

The relationship between *Knotted* genes and abnormality in oil palm development has been the subject of considerable discussion. Oil palm is a major crop species producing high quality oil used in food. In this monocotyledononous species with a single apical meristem, clonal propagation through tissue culture has began as early as 1960s since vegetative propagation of elite adult palm has proved impossible. Although extensive research from the 1980s has been successful in setting up and optimizing large-scale tissue culture propagation, but until today it still face the problems due to the presence of vegetative abnormalities within the *in vitro* plantlets as well as flowering abnormality. To date, there is no fundamental understanding of the cause of the abnormality or the methods in how to prevent the abnormality from occurring (Kubis *et. al.*, 2003).

Plant regeneration from *in-vitro*-cultured plant tissues generally involves proliferation of cells with defined developmental fates, therefore gene critical to cell division and development will likely be very useful in investigating the molecular mechanism of *in-vitro* plant (Frugis *et al.*, 1999). The objectives of this study were therefore; to isolate the full-length of *Knotted* gene from oil palm by screening an oil palm suspension culture cDNA library and to characterize this gene by studying the RNA or gene expression patterns in various tissues of oil palm using semi-quantitative PCR, northern blotting and *in situ* hybridization.